AMRL-TR-79-25

ADA 068620
citation

CONTINUOUS FLOW AQUATIC TOXICOLOGY TESTING USING DILUTION WATER BY REVERSE OSMOSIS

J. W. FISHER
R. C. INMAN
M. A. HAGERMAN
C. B. HARRAH

APRIL 1979

20060706053

Approved for public release; distribution unlimited.

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Documentation Center should direct requests for copies of this report to:

Defense Documentation Center
Cameron Station
Alexandria, Virginia 22314

TECHNICAL REVIEW AND APPROVAL

AMRL-TR-79-25

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

A. A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

AIR FORCE/56780/30 April 1979 — 100

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER AMRL-TR-79-25	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) CONTINUOUS FLOW AQUATIC TOXICITY TESTING USING DILUTION WATER BY REVERSE OSMOSIS		5. TYPE OF REPORT & PERIOD COVERED Technical Report
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) J.W. Fisher M.A. Hagerman R.C. Inman C.B. Harrah		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS AEROSPACE MEDICAL RESEARCH LABORATORY, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62202F; 6302-04-18
11. CONTROLLING OFFICE NAME AND ADDRESS Same as Block 9		12. REPORT DATE April 1979
		13. NUMBER OF PAGES 14
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Supported in part with Laboratory Director's Funds (FY78)

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Aquatic Toxicity Testing
Proportional Diluters
Reverse Osmosis

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This paper describes the design and operation of a continuous-flow aquatic toxicity testing system. Aquatic organisms are exposed to toxic chemicals in exposure tanks in which fresh water plus toxicant is continually being replenished. The water used to dilute the test chemical is well water treated by reverse osmosis filtration. The effluent is treated prior to discharging the waste water system. Specific system design considerations are discussed in detail.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

PREFACE

This study was conducted in the Toxic Hazards Division, Environmental Quality Branch, Aerospace Medical Research Laboratory. The research was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations", Task 04, Workunit 18, from April 1978 to September 1978. This work was partially supported by funds provided under FY78 Laboratory Directors Fund.

The authors wish to thank Mr. Quenton Pickering of the Environmental Protection Agency, Lieutenant Commander Morris Cowan, Mr. Bob Jones, and Dr. Melvin Andersen of the Navy Medical Research Institute/TD, Mr. Tom Poling and Dr. Dan Benz of the University of California, Mr. David Geiger, Sgt. John Bowles and all other branch personnel supporting the effort to build the toxicity testing system.

INTRODUCTION

The purpose of this paper is to furnish a descriptive document of the equipment and techniques used in aquatic toxicity studies at the Environmental Quality Branch of the Hazards Division, Aerospace Medical Research Laboratory. In these studies, aquatic organisms are exposed to toxic wastes and chemicals in exposure tanks in which the fresh water plus toxicant is continually being replenished. Experiments are designed to establish dose-response relationships of aquatic organisms to logarithmically or geometrically spaced concentrations of potential pollutants. Exposure concentrations are produced using a proportional diluter (Mount and Brungs, 1967; Lemke et al., 1978). The water used to dilute the test chemical is well water that has been treated using a reverse osmosis filtration process which controls the concentration of dissolved solids in the water. Each concentration of the test chemical flows from the diluter to an individual glass or plexiglass tank used as an exposure chamber. In each exposure chamber the aquatic organisms are observed to determine quantal or behavioral responses to that particular concentration of test chemical and the changes that occur as the length of exposure time increases. Each major aspect of the experimental design is described in detail.

METHODS

Dilution Water

The composition of the water in which aquatic organisms are held and exposed plays a critical role in biological responses to toxic contaminants. Bioassay results can be affected by many variables; possibly the most important is water quality (Slooff and Spierenburg, 1978). For example, hardness of the water may affect how rapidly or completely a pollutant is absorbed by the organisms. Natural components of water may affect pollutant concentration by binding with the pollutant or altering its rate of decomposition. The water quality of the tap water (Table 1) of building 433, Wright-Patterson Air Force Base, has a $CaCO_3$ hardness in the range of 500-600 mg/liter with intermittent large quantities of suspended iron. These two parameters of the water quality present serious mechanical, chemical, and biological problems if unaltered. Without treatment most portions of the diluter and aquaria exposed to untreated water would be covered with precipitates to an unusable extent within a few weeks. Consequently, a reverse osmosis (R.O.) membrane filtering system (Figure 1) has been installed to improve the water quality for toxicity testing. The water for the R.O. membrane is pretreated with two disposable 1.0 micron rope filters (Filterite)®, a charcoal bed, and a water softener.

TABLE 1

TAP WATER ANALYSIS BY ATOMIC ABSORPTION

Cation	ppm	Cation	ppm
Ca	100	Cu	< 3
Mg	30	Fe*	< 1
Na	21	Ni	< 3
K	4	Zn	< 0.5
Al	<10	Cd	< 0.5
Cr	< 4	Hg	< 0.2
Co	< 1	Ag	< 1.0

Dissolved Solids 79.6 mg/100mℓ
* Suspended particles are not measured

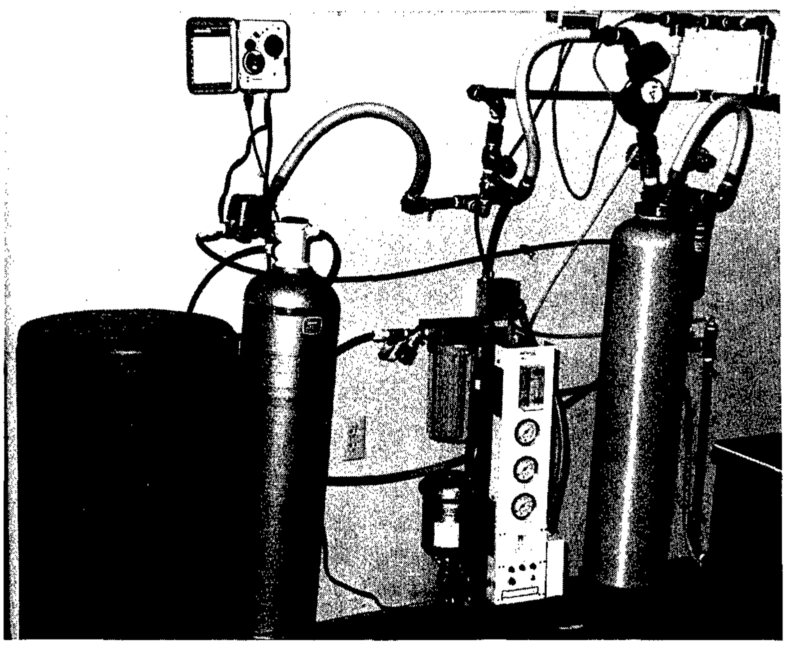

Figure 1, Reverse osmosis membrane with water softener, charcoal bed and two 1-micron rope filters.

Approximately 50 percent of the pretreated water is pumped through the R.O. membrane; the remaining 50 percent by-passes the membrane and contains the rejected dissolved solids of the water that did pass through the membrane. The R.O. membrane is capable of at least 90% rejection of total dissolved solids. This particular unit (Continental Model 3016) has a nominal rating of 800 gallons of treated water per day at 77°F. This system presently produces approximately 0.7 gallons per minute (gpm) of 0.03-0.05 megaohm water. This means the rejection of total dissolved solids is 98-99%. In order to produce the desired hardness, the treated water of the R.O. system is diluted with charcoal treated tap water. During experimental tests the usual electrolyte content (NaCl) is 116-170 ppm.

The treated (R.O.) water mixed with the tap water after organic removal is stored in a 130 gallon "Living Stream"® tank. The water level in the reservoir is electrically controlled by activation or deactivation of a float switch. A safety overflow pipe is used to detour the water to a floor drain in case of an accidental overflow. A "Little Giant"® submerged pump in the reservoir attached to ¼" (I.D.) stainless steel tubing is used to deliver the conditioned water to the proportional diluter. A stainless steel needle valve inline between the pump and the diluter regulates the flow rate of the water. The volume of the conditioned water in the reservoir must be large enough to maintain an adequate supply for the diluter during two periods, when the R.O. is not treating water during daily water softener regeneration (one hour) and when the R.O. membrane is flushed (30 minutes) by increasing the percentage of water by-passing the membrane. The treated water dwindles to approximately 0.2 gpm. These two operations can be performed manually or automatically.

Proportional Diluter

Lemke et al. (1978) in a recent paper describe the proportional diluter operation, construction, and calibration. Diluter modifications such as safety devices, toxicant delivery systems, and toxicant metering systems are also discussed. The paper helps clarify and provide modifications to Mount and Brungs (1967) original publication on diluter fabrication. The construction of the diluter used in this laboratory was a compromise between these two systems together with other modifications made by the laboratory. Only the modifications to the diluter designed by this laboratory was presented. The proportional diluter (Figure 2) cell volumes are equivalent to the Lemke et al. design; however, the siphons for the cells are designed as described by Mount and Brungs except for the use of glass bubble breaks rather than polyethylene bottles, and a 250 mℓ polyethylene bottle for the bubble-break below the top left cell (W-1).

One-fourth inch plexiglass (instead of glass) was used to construct the diluter cells, with the exception of a seven liter glass jar (M) used to contain the water and toxicant below the W-1 cell. The frame was built of unistrut steel and 3/4" plywood for support of the exposure aquaria and diluter cells.

The electrical wiring to the proportional diluter (Figure 3) is housed in conduit and connection boxes. A 125 mℓ polyethylene bottle attached with string to a microswitch regulates the cycling time; as the water fills the valve bucket (located below and to the right of top right cell of the diluter), the bottle rises, deactivating the microswitch, thus halting the treated water entering the W-1 cell

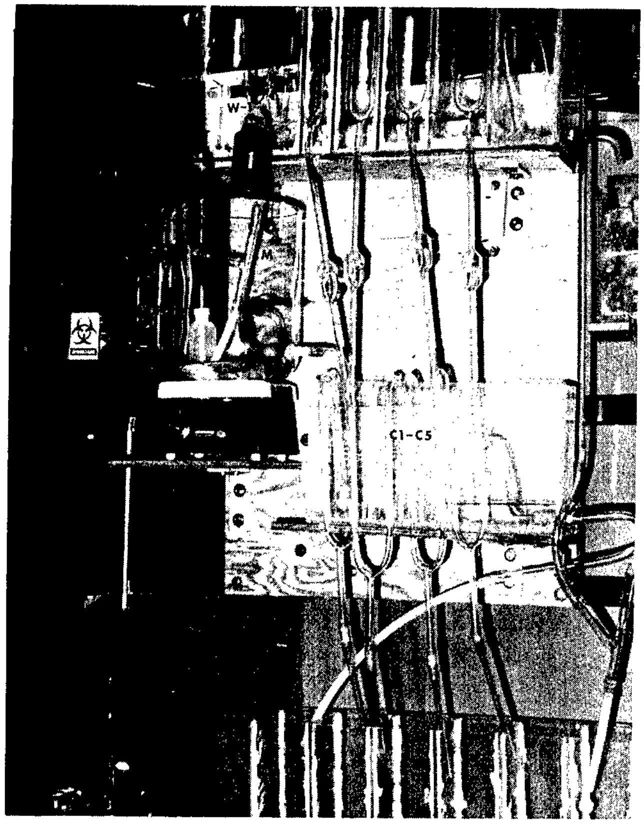

Figure 2. Proportional Diluter

The toxicant delivery system is a Hamilton® precision dispenser (Figure 4) activated by 20-30 pounds per square inch of compressed air. This dispenser can deliver from one microliter to ten milliliters of neat toxicants into the mixing chamber cell (M), located directly below the W-1 cell. The M cell with the W-1 cell water and the diluted toxicant then fills the cells C1 to C5, located directly below and to the right of the M cell.

A 125 mℓ polyethylene bottle is located in the M cell with a 1/8" x 12" stainless steel rod attached to the top of the bottle. When the water flow from cell W-1 fills cell M, the bottle rises with the attached rod. The guide for the rod, a 1/4" (I.D.) x 10" tube is used to align the rising rod with a sensitive valve (i.e. automotive tire valve) located at the end of the guide. The valve is located inside the tygon tubing and the tygon tubing is attached to the rod guide. Consequently, when the water in M cell rises, the bottle and the rod inside the guide also rise, triggering the dispenser as the rod presses against the valve.

Exposure Aquaria

The receiving aquaria consist of six 30 liter aquaria (Figure 5) on one side of the diluter and six 9-liter aquaria (Figure 6a and 6b)

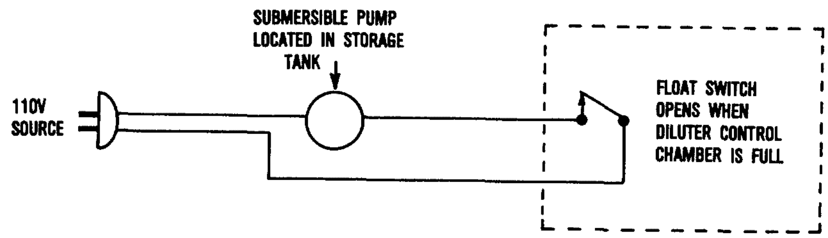

Figure 3. Schematic of the electrical system for the water delivery to the proportional diluter.

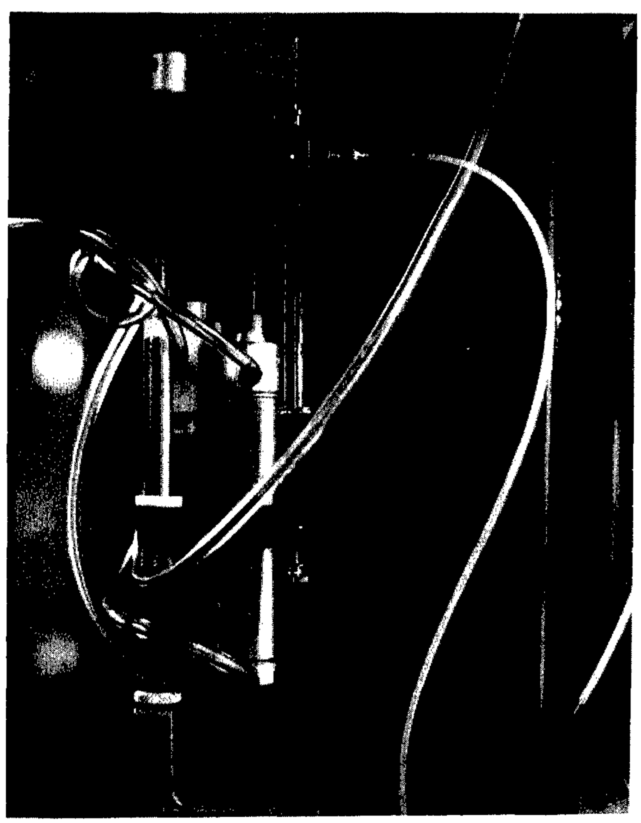

Figure 4. Hamilton precision dispenser used for the toxicant delivery.

on the other side. The smaller aquaria are used for behavioral observations (Vinyard and O'Brian, 1975). To adjust for differences in partial replacement time due to differing volumes, stainless steel elbows were installed at the end of the receiving tubes of the behavioral aquaria to reduce the flow rate. This provides a flow ratio of approximately 2:1 for the large and smaller aquaria, respectively. All aquaria are fabricated with 1/4" plexiglass.

Effluent Disposal

The effluent from the exposure tanks is drained into a 15-gallon plexiglass decontamination aquarium via 2" polyvinylchloride pipe. A separatory funnel placed over this aquarium provides a constant delivery of chemicals (i.e. sodium hypochlorite) to the aquarium to decontaminate the effluent from the exposure tanks. The decontaminated effluent is siphoned to the floor drain. The approximate retention time for the effluent from the exposure tanks and decontaminant from the separatory funnel in this aquarium is 15 minutes.

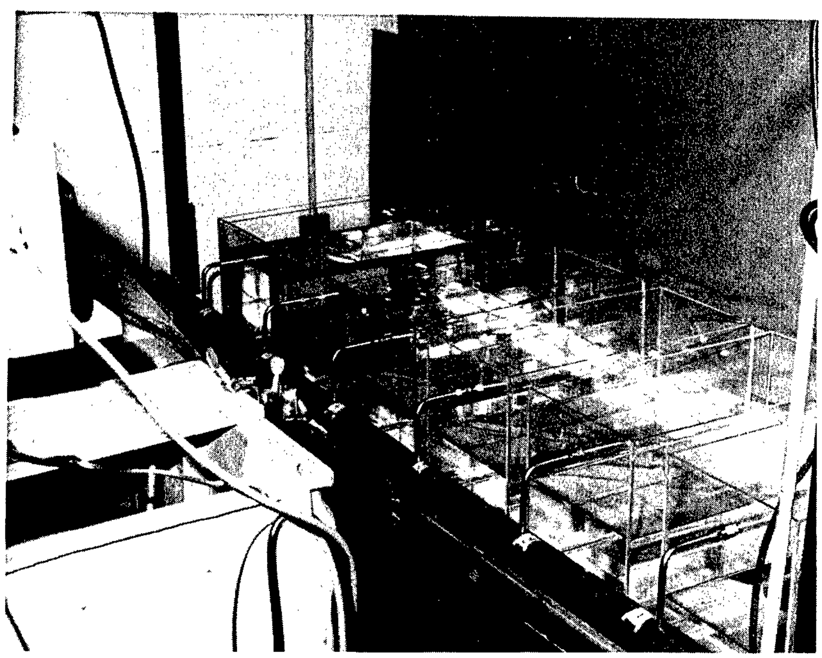

Figure 5. The 30 liter aquaria used for quantal responses.

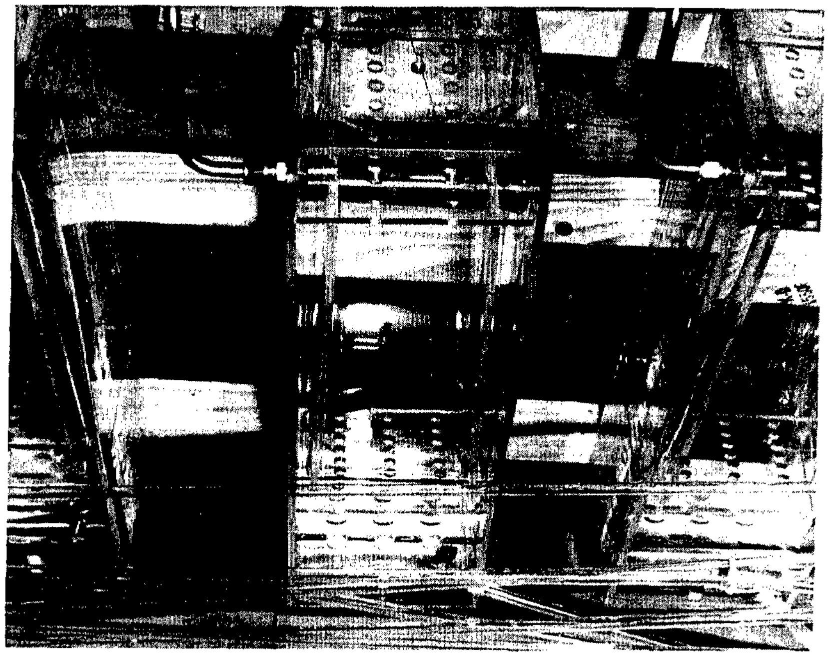

Figure 6a. Behavioral response aquaria.

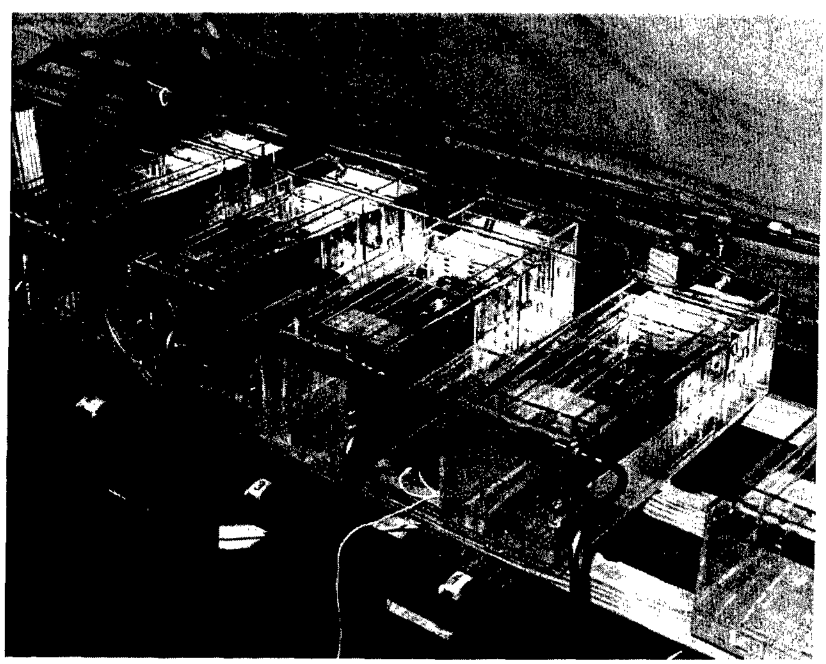

Figure 6b. Front view of behavioral response aquaria showing construction design.

DISCUSSION

This system was designed for limited use in an aquatic toxicology laboratory. The floor space and water quality can become limiting factors, depending on the amount of water used, cost to produce the water, and methodology used to clean the water (Figure 7). The pretreatment of the water before the R.O. membrane may require the addition of a flocculating agent and/or sand filtration before organic removal and water softening. Possible use of an ultrafiltration unit should filter particles 0.0005 microns or larger. Pretreatment of the water before the R.O. membrane is essential because divalent cations or chlorine will cause irreversible damage to the R.O. membrane. Long term use of the R.O. with periodic cleaning of the membrane may still yield reduced quality of water that has been attributed to a foulant coating containing 70% organic matter (Cadotte and Peterson, 1977).

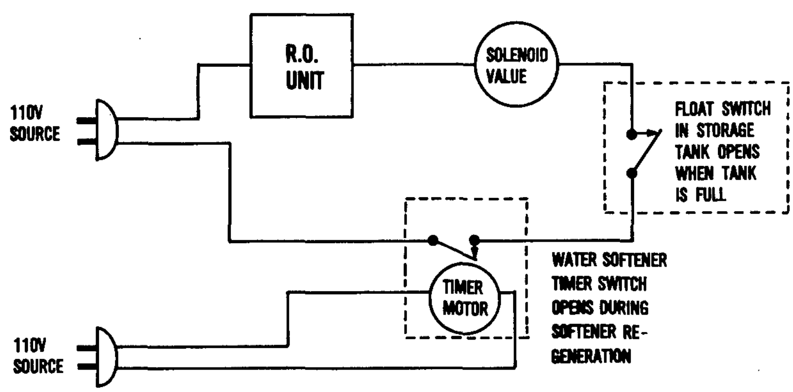

Figure 7. Electrical schematic of R.O. system and reservoir design.

REFERENCES

Cadotte, J.E. and R.J. Peterson 1977, Mechanism and prevention of reverse osmosis membrane fouling, ARO 14-93, 1 CX, U.S. Army Research.

Gregor, C.D. and H.P. Gregor, 1978, Synthetic membrane technology, Scientific American 239 (1): 112-128.

Lemke, A.E., W.A. Brungs and B.J. Halligan, 1978, Manual for construction and operation of toxicity-testing proportional diluters, EPA 600/3-78-072.

Mount, D.I. and W.A. Brungs, 1967, A simplified dosing apparatus for fish toxicology studies, Water Research 1:21-29.

Slooff, W., E. Rab, and T.P. Spierenburg, 1978, Continuous standard water delivery system for bioassays with aquatic organisms, The Prog. Fish Cult. 40 (3): 112-114.

Vinyard, G.L. and W.J. O'Brian, 1975, Dorsal light response as an index of prey preference in bluegill (Lepomis macrochirus). J. Fish Res. Bd. Can. 32(10): 1860-1863.

☆U.S.Government Printing Office: 1979 — 657-002/569